Central metabolic pathway

Overview

Aerobic oxidation of glucose

- **Glycolysis** - Glucose oxidised to 2 Pyruvate. Formation of 2 NADH and 2 ATP via substrate level phosphorylation.

- **Pyruvate dehydrogenase (PDH)** - Pyruvate oxidised to Acetyl-coA. Formation of 1 NADH and 1 CO₂.

- **TCA cycle** - Acetyl-coA reacts with oxaloacetic acid (OAA) to form citrate. One "turn" of TCA cycle oxidises completely the Acetyl group and generates 2 CO₂, 3 NADH, 1 FADH and 1 ATP (or 1 GDP) via substrate level phosphorylation. End product is OAA, which can react with another Acetyl-coA.

- **Electron transport chain** - NADH, FADH reoxidised to NAD, FAD. Electrons are transferred in the electron transport chain towards an increasing reduction/redox potential, which releases energy. Part of this energy is used to pump protons across a membrane against its gradient

  => generation of proton motive force (PMF) consisting of

  1) concentration gradient H⁺

  2) Charge difference (outside positive, inside negative)

  Protons are moving with their gradient through ATP:ase (an enzyme), energy released will be used by ATP:ase to produce ATP. Electrons are finally transferred to O₂, which will be reduced to H₂O. This is called "Mitchell's chemiosmotic mechanism."
Anaerobic oxidation of glucose

- Glycolysis - same as aerobic.
  Due to limited supply of NAD/NADH, NADH needs to be reoxidised, otherwise glucose oxidation would be blocked in the absence of electron acceptor → fermentation

**Example:** Fermentation: $\text{Pyruvate} \overset{\text{NAD}}{\rightarrow} \text{Acetaldehyde} \overset{\text{CO}_2}{\rightarrow} \text{Ethanol}$

  $\text{Pyruvate} \overset{\text{NAD}}{\rightarrow} \text{Lactate}$

We will look more into details of glycolysis, gluconeogenesis, glycogen metabolism, pentosephosphate pathway (Chapter 12)

**Glycolysis** (takes place in the cytosol)
Consists of 10 reactions, see fig 12.4

Most important reactions for rate control are carried by hexokinase, phosphofructokinase, pyruvate kinase. The flux is also controlled by biosynthesis and degradation of storage carbohydrates as well as transport of glucose across cell membrane.

1) **Hexokinase** (ATP dependent phosphorylation of glucose)

Glucose + ATP $\rightarrow$ Glucose - 6-phosphate + ADP

Irreversible process (see Table 12.1) $\Delta G \approx -33.5 \text{ KJ/mole}$

($\Delta G$ is highly dependent on concentration, in the cell: Concentration of Glucose - 6-phosphate will never be that high to make irreversible reaction possible.)

Many different forms of hexokinase in different organisms and tissues: isoenzymes = enzymes carrying out the same reaction, but small difference in amino acid composition will cause difference in e.g. kinetics, co-enzyme, specificity.

Hexokinase I, II, III & IV in mammals, see fig p.411; different kinetics.
2) **Glucose-6-phosphate isomerase** (isomerization of G6P)
   (Sometimes called phosphoglucoisomerase)
   \[ \text{G6P} \rightleftharpoons \text{Fructose-6-phosphate} \quad \Delta G \approx -2.5 \text{kJ/mole} \]

3) **Phosphofructokinase** (ATP dependent phosphorylation of F6P)
   \[ \text{F6P} + \text{ATP} \rightarrow \text{Fructose-1,6-bisphosphate} + \text{ADP} \]
   Irreversible, \( \Delta G \approx -22 \text{kJ/mole} \)
   Very important rate regulatory enzyme. Many affector molecules.
   Inhibited by ATP, citrate. Activated by ADP, AMP, (in some cell NH₄⁺),
   Fructose-2,6-bisphosphate. Energy charge (Ec) affects rate,
   \[ Ec = \frac{[\text{ATP}]}{[\text{ATP}]+\frac{1}{4}[\text{ADP}]+[\text{AMP}]} \]
   via individual components not by Ec itself.
   Synthesis/degradation of F2.6P via a bifunctional protein that
   acts as a kinase as well as phosphatase
   a) Form F2.6P from F6P kinase activity
   b) Degrade F2.6P to F6P phosphate activity
   In mammals, there are different isoenzymes with different kinetics/
   /regulation in different cell types.

4) **Fructose-1,6-bisphosphate aldolase** (cleavage of F1,6P to two triosephosp
   (Fructose-1 = enzyme is often just referred to as aldolase)
   \[ \text{F1,6P} \rightleftharpoons \text{Dihydroxyacetonephosphate} + \text{Glyceraldehyde-3-phosphate} \]
   "DHAP" "GAP"
   Reversible process, but \( \Delta G^o = +23.9 \text{kJ/mole} \), concentration of DHAP
   and GAP is very low compared to F1,6P \( \Rightarrow \Delta G < 0 \) for cellular conditions

5) **Triosephosphate isomerase** (isomerisation of DHAP)
   \[ \text{DHAP} \rightleftharpoons \text{GAP} \quad \text{Reversible process} , \Delta G \approx 0. \]
   Reaction 1-5 is called "investment" phase or upper part of glycolysis.
   1 Glucose (6 carbon) consumed and formation of 2 GAP (3c) and
   consumption of 2 ATP.

6) **Glyceraldehyde-3-phosphate dehydrogenase** (oxidation and formation
   of high energy compound)
   \[ \text{GAP} + \text{NAD} + \text{Pi} \rightleftharpoons 1,3-\text{bisphosphoglycerate} + \text{NADH} \]
   Reversible process, \( \Delta G \approx 0 \). 1,3-BPG: "high energy compound"
7) **Phosphoglycerate Kinase** (formation of ATP via substrate level phosphorylation)
   \[ 1,3\text{BPG} + \text{ADP} \rightleftharpoons 3\text{-Phosphoglycerate} + \text{ATP} \]
   Reversible process, \( \Delta G \approx 0 \). But \( \Delta G^0 \) large negative value => “drives” the reaction to the right keeping \([1,3\text{BPG}]\) very low, which is a prerequisite for reaction 6 with a high positive \( \Delta G^0 \) to proceed in the correct direction. Reaction 7 “drives” reaction 6.

8) **Phosphoglycerate mutase** (isomerisation of 3PG)
   \[ 3\text{PG} \rightleftharpoons 2\text{-Phosphoglycerate} \]
   Reversible, \( \Delta G \approx 0 \)

9) **Enolase** (2PG converted to high energy compound)
   \[ 2\text{PG} \rightleftharpoons \text{Phosphoenolpyruvate “PEP”} \]
   Reversible, \( \Delta G \approx -2.5 \text{kJ/mole} \)

10) **Pyruvate Kinase** (formation of ATP via substrate level phosphorylation)
    \[ \text{PEP} + \text{ADP} \rightarrow \text{Pyruvate} + \text{ATP} \]
    Irreversible, \( \Delta G \approx -17 \text{kJ/mole} \)
    Rate regulating step. Many different isoenzymes with in part different regulation in different tissues. Most are allosterically activated by F1.6P (feed forward mechanism) and inhibited by ATP, Acetyl-coA.

    | The name *Kinase* indicates that reaction is taking place in the other direction. |
Gluconeogenisis

Formation of carbohydrates from other substances.
Such as lactate, aminacids, glycerol from degradation of lipids.
But mammals can’t produce carbohydrates in the absence of the glyoxylate cycle.
Glucose essential for some organs such as the brain.
Glucose / Glucose-6-P precursors for other essential metabolic pathways.
Gluconeogenisis = reversal of glycolysis but irreversible reactions are by passed by synthesising alternative enzymes.
See fig 12.10

Hexokinase, phosphofructo kinase and Pyruvate kinase are not active during gluconeogenisis.
Regulation of glycolysis and gluconeogenisis is reciprocal, i.e. factors that stimulate gluconeogenisis will inhibit glycolysis and vice versa. To avoid so called futile cycles (wasteful degradation of ATP).
Regulation by metabolite levels, ATP, AMP, F2.6P but also by hormones glucagone insulin.

1) Bypass Pyruvate $\rightarrow$ Phosphoenolpyruvate (PEP)
   Two enzymes 1. Pyruvate Carboxylyks
   2. Phosphoenolpyruvate carboxykinase (PEPck)
   Pyruvate + CO$_2$ + ATP $\rightleftharpoons$ Oxaloacetatic acid + ADP + P$_i$
   Pyruvate carboxylase localized in mitochondria (Matrix) OAA
   TCA- cycle intermediate OAA is reduced to Malate by
   Malate dehydrogenesase, NADH is consumed and Malate is exported to the cytosol where it is oxiredised to OAA by a cytosolic
   malate dehydrogenesase.
   PEPck : OAA + GTP $\rightleftharpoons$ PEP + CO$_2$ + GDP
2) Bypass $F\text{1.6P} \rightarrow F\text{6P}$
Fructose-1,6-bisphosphatase catalyses a reaction that is not reversal of phosphofructokinase
$F\text{1.6P} + H_2O \rightarrow F\text{6P} + P_i$

3) Bypass $G\text{6P} \rightarrow$ Glucose by glucose-6-phosphate
$G\text{6P} + H_2O \rightarrow$ Glucose + $P_i$

Futile cycles ex.

\[
\text{phosphofructokinase ATP} \rightarrow F\text{6P} \rightarrow P_i \rightarrow \text{Fructose 1.6 bisphosphatase}
\]

\[
F\text{1.6P}
\]

**Fermentation**

Glucose $\rightarrow 2$ Pyruvate, $2NADH, 2ATP$

Limited amount of $NAD/NADH \rightarrow NADH$ must be reoxidised in order to continue the process.

Aerobically $\Rightarrow$ mitochondrial $e^-$-transport

Anaerobically $\Rightarrow$ Fermentation

**Fermentation** = Redox neutral process, no net oxidation or reduction in the absence of external $e^-$-acceptor and ATP is produced.

"Rearrangements of existing molecules"

Ex: lactate formation

$\text{Pyruvate} + NADH \rightarrow \text{lactic acid} + NAD$

Lactate dehydrogenase.

Ex: Ethanol formation in baker's yeast Saccharomyces ceravisiae

$\text{Pyruvate decarboxylase (PDC)}$

Alcohol dehydrogenase (ADH)

$\text{Pyruvate} \xrightarrow{\text{PDC}} \text{Acetaldehyde} \xrightarrow{\text{ADH}} \text{Ethanol}$
Electron balance

Pyruvate more oxidised than glucose
Lactic acid has the same oxidation level as glucose
Ethanol is more reduced than glucose

The more reduced a compound is the more energy it contains.
The extent of reduction of a compound can be calculated by its degree of reduction (δ)

\[ \delta = \text{available electrons per } \text{C-mole of a compound} \]

1 C-mole = 12 g or 1 mole C

1 mole glucose (C₆H₁₂O₆) contains 6 C-mole or 72g C.

Calculation of δ by C = 4, H = 1, O = -2
Ex: Glucose C₆H₁₂O₆, C₄H₂O (C-mole formula)
\[ \delta = 4 + (2 \cdot 1) + (1 \cdot 2) = 4 \quad \text{or} \quad \delta = \frac{6 \cdot 4 + (2 \cdot 1) + (1 \cdot 2)}{6} = 4 \]

Ex: Ethanol C₃H₇OH, C₄H₂O₇/₂ \[ \delta = 4 + (3 \cdot 1) + \left( \frac{1}{2} \cdot (-2) \right) = 6 \]
Ex: CO₂ \[ \delta = 4 + (2 \cdot -2) = 0 \quad \text{Most oxidised form of C.} \]
Ex: CH₄ \[ \delta = 4 \quad \text{Most reduced form of C.} \]

you can't have more than 8 electrons in the outer shell.

Ethanol formation

\[ \text{C₆H₁₂O₆} \rightarrow 2 \text{C₃H₇OH} + 2 \text{CO₂} \]
\text{mass balance ok!}

\[ \begin{align*}
\text{e}^-\text{-balances} & : & \ 6 \cdot 4 & : & \ 4 \cdot 6 & : & \ 2 \cdot 0 \\
\text{平衡} & : & \ 24 & : & \ 24 & : & \ 0
\end{align*} \]

One part of glucose reduced to ethanol another part oxidised to CO₂ \[ \Rightarrow \text{no net oxidation} \]
Usage of other carbohydrates than glucose

Monosaccharides such as galactose, mannose and fructose are used and converted to different intermediate in glycolysis. See fig. 12.19

Disaccharides such as Maltose, Lactose, Sucrose and so on.

Metabolism of starch and Glycogen

Hydrolysis of starch and glycogen in the mouth and gut performed by amylase and glucosidase \(\Rightarrow\) Maltose + Glucose

Mobilization of intracellular glycogen

Phosphorylation via:

1) Glycogen phosphorylase breaking \(\alpha(1-4)\) bonds until it reaches 4 glucose units before a \(\alpha(1-6)\) bond (branch-point).

Results in formation of Glucose-1-phosphate.

2) Glucan transferase (Debranching enzyme)
   a) breaks \(\alpha(1-4)\) bond and transfers the resulting trisaccharide to a nearby free end and joins again with a \(\alpha(1-4)\) bond \(\Rightarrow\) glycogen phosphorylase can act again; see fig 12.22
   b) breaks \(\alpha(1-6)\) bond. Results in glucose

Glucose-1-phosphate converted by phosphoglucomutase to Glucose-6-phosphate

Bioynthesis of Glycogen

See fig 12.23

1) UDP-glucose pyrophosphorylase forms activated glucose.
   
   \[\text{G}_1\text{P} + \text{UTP} \rightarrow \text{UDP-glucose} + \text{P}_1; \quad \text{------} \rightarrow \text{P}_2;\]
   
   "UDPG"

2) Glycogen synthase
   
   Glycogen \(_\text{Gkm}\) + UDPG \(\rightarrow\) Glycogen \(_\text{Gkm}\) + UDP

But glycogen \(_\text{Gkm}\) must consist of at least 4 glucose units, produced by (3)
3) Glycogenine
   Glycogenin + UDPG → Glycogenin - Glc (glucose molecule binds a tyrosine unit on the enzyme)
   Glycogenin - Glc + UDPG → Glycogenin - Glc - Glc etc up to 8 glucose units.

4) "Branching enzyme" (amylo -(1,4 → 1,6) transglycolase) introduces branch points.
Regulation of Glycogenphosphorylase, see fig. 12.26
Epinephrine (adrenaline) binds receptor on cell surface.
⇒ Adenylate cyclase activated via a G-protein (GTP activated protein) and C-AMP is formed from ATP.

In addition: Inactive Glycogenphosphorylase b can be activated allosterically via high levels of AMP (starvation condition).
Phosphorylase b kinase contains Calmodulin (=Ca^{2+} modulating protein)
⇒ this kinase is activated by Ca^{2+}.

Regulation of Glycogenbiosynthesis (Glycogen synthase)
Reciprocal (ömsesidig) regulation degradation - biosynthesis of Glycogen.
Glycogen synthase inactive in phosphorylated form. Glycogen synthase phosphorylated directly by PKA, but also by many other protein kinases
⇒ different phosphorylation pattern an forms of Glycogen Synthase.
Inactive phosphorylated form of glycogen synthase can be activated allosterically by high levels of G6P.
Phosphoproteinphosphatase (PPi) dephosphorylates and activates glycogen synthase.
Pentose phosphate pathway, see fig 12.32 (PP pathway)
Located in the cytosol, two main functions:
1) Formation of reducing power NADPH
2) Formation of ribose-5-phosphate, precursor for biosynthesis of nucleic acids and nucleotides.

Can be divided into two parts, oxidative and non-oxidative, respectively:

**Oxidative part**  See fig 12.33

\[
\text{Glucose-6-phosphate} \xrightarrow{\text{NADPH}} \text{6-phosphogluconolactone} \xrightarrow{\text{Glucose-6-phosphate dehydrogenase}} \text{CO}_2
\]

The ribulose-5-phosphate will carry out this part:

**Non-oxidative part**

\[
\text{Ribulose-5-phosphate} \rightarrow 2 \text{Fructose-6-phosphate} + 1 \text{Glyceraldehyde-3-P}
\]

\[
\Sigma 3\text{G6P} + 6\text{NADP} \rightarrow 2\text{F6P} + 1\text{GAP} + 6\text{NADPH} + 3\text{CO}_2
\]

PP pathway can be adjusted in 3 different ways depending on requirements:
See fig. 12.36 → 1) nucleotides, 2) NADPH, 3) energy

1. Nucleotide/nucleic acid biosynthesis ⇒ only oxidative part.
   Ribulose-5-P transformed into Ribose-5-P.

2. NADPH generation ⇒ both oxidative and non-oxidative part.
   GAP + F6P via gluconeogenesis back to G6P and everything starts over again.

3. ATP production ⇒ both parts, GAP and F6P further oxidised in glycolysis, PDH and TCA-cycle

Regulation of PP pathway via NADP/NADPH ratio, which effects activity of Glucose-6-phosphate dehydrogenase.
Pymvate dehydrogenase, TCA and Glyoxylate cycle (Ch.13)

Degradation of organic material can be divided in 3 steps, fig 13.2
1) Formation of 2-carbon fragments, acetyl group of Acetyl-CoA.
   Different routes for carbon hydrates, proteins (see fig. 18.12) and
   lipids (see fig. 16.12)
2) Oxidation of acetyl group in TCA-cycle and generation of NADH, FADH
3) Reoxidation of NADH and FADH in e^- trp chain

Pyrurate dehydrogenase (PDH)
Large protein complex consisting of 3 enzymes and 5 co-enzymes
Pymvate + NAD + CoA - SH → Acetyl-CoA + NADH + CO2
Regulation of activity via feed-back inhibition by Acetyl-CoA
and NADH as well as covalent modification (phosphorylation)
Pyrurate dehydrogenase kinase phosphorylates and inactivates PDH.
PDH-phosphatase dephosphorylates and activates PDH.
Kinase activated by NADH, Acetyl-CoA and ATP ⇒ PDH inactive
Kinase inhibited by ADP, pyruvate ⇒ PDH active
Phosphatase is activated by Ca**, Mg** and insulin ⇒ PDH active.

TCA-cycle
In the matrix of mitochondria.
Most important regulation by NAD/NADH ratio. Overview, see fig. 13.4
1) Citrate synthase (incorporation of 2 carbon from Acetyl-CoA)
   (often without use of ATP, Synthetase - requires ATP - most cases)
   \[ \text{Acetyl-CoA + OAA + H}_2\text{O} \rightarrow \text{Citrate + CoA - SH} \]
   \[ \text{C}_2 \quad \text{C}_4 \quad \text{C}_6 \]
   \[ \Delta G^{\circ} = -22 \text{ kJ/mole}, \text{ but at cellular concentration } \Delta G \approx 0. \]
   Concentration of OAA very low.
This is a prerequisite (nödvändig förutsättning) for reaction 8 to proceed
(Which has a very positive \( \Delta G^\circ \) ⇒ "Reaction 1 drives reaction 8", see
   Table 13.2 (OBS \( \Delta G \) for reaction 1 is not correct in table!)
Inhibited by NADH and NADPH as well as succinyl-CoA.)
2) Aconitase (isomerization of citrate)
\[ \text{Citrate} \rightarrow \text{aconitate} \rightarrow \text{isocitrate} \quad \Delta G^\circ = +6.3 \text{ kJ/mole} \]
but next reaction has a rather negative \( \Delta G^\circ \) value keeping isocitrate concentration low and hence, \( \Delta G \) for aconitase is < 0.
Inhibited by fluoroacetate (rodenticide)

3) Isocitrate dehydrogenase (oxidative decarboxylation)
\[ \text{Isocitrate} + \text{NAD} \rightarrow \alpha \text{-ketoglutarate} + \text{NADH} + \text{CO}_2 \]
Allosteric regulation, inhibited by NADH and ATP, activated by ADP.

4) \( \alpha \)-ketoglutarate dehydrogenase (oxidative decarboxylation)
\[ \alpha \text{-ketoglutarate} + \text{NAD} + \text{CoA} \cdot \text{SH} \rightarrow \text{Succinyl-CoA} + \text{NADH} + \text{CO}_2 \]
Inhibited by NADH and succinyl-CoA. Allosteric activation by Ca\(^{2+}\).

Reaction 3) and 4) are key enzymes in rate regulation of TCA cycle. Acetyl group oxidised to \( 2\text{CO}_2 \) and NADH is generated. \( \alpha \)-ketoglutarate key compound during biosynthesis and degradation of amino acids.

5) Succinyl-CoA synthetase (formation of ATP/GTP by substrate level phosphorylation).
\[ \text{Succinyl-CoA} + \text{Pi} + \text{ADP} \rightarrow \text{Succinate} + \text{ATP} + \text{CoA} \cdot \text{SH} \]

6) Succinate dehydrogenase (oxidation, formation of FADH)
\[ \text{Succinate} + \text{FAD} \rightarrow \text{Fumarate} + \text{FADH} \]
Enzyme is bound to inner membrane of mitochondria and is also part of e\(^{-}\)-trp chain (usually referred to as Complex II).

7) Fumarate hydratase (fumarase) (hydration of C=O bond)
\[ \text{Fumarate} + \text{H}_2\text{O} \rightarrow \text{L-Malate} \]

8) Malate dehydrogenase (oxidation of Malate)
\[ \text{L-Malate} + \text{NAD} \rightarrow \text{OAA} + \text{NADH} \]
\( \Delta G^\circ = +29.7 \text{ kJ/mole} \), but exergonic citrate synthase reaction keeps [OAA] very low, so that \( \Delta G < 0 \).
6. Anapleurotic pathways

Intermediates in TCA-cycle are used as precursors for biosynthetic reactions – replenishing reactions are required to add intermediates to the TCA-cycle, otherwise it would stop due to a lack of OAA, see fig. 13.17 (do not need to know details)

Glyoxylate cycle

Anabolic version of TCA-cycle. Many reactions similar to TCA-cycle, but CO₂ generating reactions are replaced and the functions completely different. Exists in microorganisms and plants, not in mammals. Enables synthesis of carbohydrates from lipids and growth of two carbon compounds. See fig. 13.18

Two unique enzymes: 1) Isoyrate lyase

\[ \text{Isoyrate} \rightarrow \text{Glyoxylate} + \text{Succinate} \]

\[ C_6 \quad C_4 \]

2) Malate synthase

\[ \text{Glyoxylate} + \text{Acetyl-CoA} \rightarrow \text{Malate} \]

Cyclic process: "1 turn" incorporates 2 Acetyl-CoA and generates 1 Succinate.

Glyoxylate cycle situated in glyoxysomes succinate is exported to mitochondria, where TCA-cycle enzymes convert this to Malate which is exported to the cytosol. Malate is then oxidised to OAA and via gluconeogenesis carbohydrates are formed. See fig. 13.19

Electron transport and oxidative phosphorylation (Ch. 14)

These processes takes place in and across the inner mitochondria membrane or, in bacteria, in and across plasma membrane. Inner mitochondria membrane is very protein rich (70-80% proteins). Energy released during oxidation-reduction processes: \( \Delta G = -n F \Delta E \)

In higher organism channels e⁻ to NADH and FADH, which acts as e⁻-donors with O₂ as e⁻-acceptor. NAD/NADH has a more negative reduction potential compared to FAD/FADH. \( \Rightarrow \) More energy released with NADH as e⁻-donor than FADH
Mitchells chemiosmotic mechanism (fig 14.2, 14.3)

(Important to know!)

Complexes and components there in are organized such that e⁻s are constantly transferred to a higher reduction potential, see fig 14.8

⇒ energy is released and part of this is used to pump protons across the membrane ⇒ generation of proton Motive Force (PMF) consisting of ① concentration difference of H⁺ and ② change difference between inside and outside, see fig. 14.17.

Energy released when protons are transferred with their concentration gradient through ATP:ase and part of energy is used to form ATP.

Components constituting the e⁻-transport chain (fig 14.9)

• Complex I (NADH dehydrogenase) transfer e⁻-s to CoQ.
• Complex II (Succinate dehydrogenase, also in TCA-cycle) transfer e⁻-s to CoQ (not Com. I → Com. II → CoQ)
• Coenzyme Q (Ubiguinin)
• Complex III (Coenzyme Q-cytochrome C oxidoreductase
• Cytochrome C
• Complex IV (cytochrome oxidase)

Inhibitors of e⁻ transport

• Rotenone, amyotal - inhibiting complex I/NADH dehydrogenase
• Antimycin A - inhibits complex III.
• Cyanide, CO, Azide - inhibits complex IV/Cytochrome oxidase.

Oxidative phosphorylation

Glycolysis: 2 ATP, 2 NADH

PDH: 2 NADH (pyruvate dehydrogenase)

TCA-cycle: 6 NADH, 2 FADH, 2 ATP / Glucose

P/O - ratio = ATP produced per O consumed or ATP per 2 e⁻ - see

fig. 14.8.

Mitchells chemiosmotic mechanism leads to two important conclusions. 1) P/O - ratio, not an integer number (could be 1,55)

2) P/O - ratio does not have to be constant

Assume P/O - ratio for NADH = 2.5 and FADH = 1.5
Complex \( V \) ATP-synthase or \( F_0F_1 \) ATP:\:ase (fig 14.18, 14.19)

\( F_0 \): part embedded in the membrane.
\( F_1 \): "krob" in matrix
\( O \): oligomycin, it blocks proton transport through \( F_0 \) and thereby ATP synthase.

**Uncouplers**: substances like 2,4-DNP, CCCP, FCCP causes the membrane to be freely permeable to protons \( \Rightarrow \) PMF collapses
\( \Rightarrow \) No ATP formation and uncoupled \( e^- \)-transport with maximum rate of respiration.

**Regulation/control of respiratory rate**

Substrate limitation via ADP, Pi, \( O_2 \), NADH/FADH

Respiratory control: \( e^-\)-transport chain and respiration is only active if ATP synthesized.

ADP stimulates and ATP inhibits for regulation.

Huge response when you add ADP
\( \Rightarrow \) isolation of mitochondria is very good.
Poor isolation \( \Rightarrow \) large response when you add NADH and small for ADP.
\( \Rightarrow \) no idea to continue experiment.

**Mitochondrial transport systems** (see fig 14.26, no details needed)

- **Adenine Nucleotide Translocase**: (ADP/ATP carrier or ANT)
  ATP out of mitochondria while ADP is going in. In this case \( Mg^{2+} \) is not bound to ATP, ADP \( \Rightarrow \) ATP\(^{4-}\), ADP\(^{3-}\) and transport is driven by proton gradient.
- **Phosphate translocase**: either antiport \( H_2 \)\(PO_4^-\) in and \( OH^-\) oct or symport \( HPO_4^{2-}\) in together with \( 2H^+\)
Redox shuttles

NADH cannot cross inner mitochondrial membrane. NADH produced in the cytosol must be transferred to matrix to be oxidised in e−-transport chain ⇒ Redox shuttles

Two systems, see fig 14.27

1) Glycerol-3-phosphate shuttles (brain, skeletal muscles)

Glucose → Glycerol-3-phosphate

\[ \text{DHAP} \quad \text{Glycerol-3-phosphate} \quad \text{GAP} \quad \text{FADH} \]

Mitochondrial G3P dehydrogenase with a catalytic site directed to the cytosol, which oxidises G3P back to DHAP.
FAD reduced to FADH. FADH oxidised in e−-transport chain.

2) Malate / Aspartate shuttles (liver, kidney, heart)

\[ \text{OAA} \quad \text{Malate} \quad \text{Malate} \quad \text{Aspartate + } \alpha\text{-keto glutarate} \]

\[ \text{NADH} \quad \text{NADH} \quad \text{OAA + Glutamate} \]

Cytosol → matrix
Photosynthesis (ch. 15) (what to know from self studies)
- Two reactions: Light and dark reactions.
- Why different photosynthetic pigments?
  - to be able to collect energy from a broad wavelength and different intensities.
- Photosynthetic system in green plants, blue green bacteria, the so called Z-scheme.
  - Two systems P680, P700
  - Where does the e's come from?
  - How is ATP produced?
  - Difference between cyclic and non-cyclic photosynthesis and which end products that are produced in each case.
  - RUBISCO protein, what reaction does it participate in?

Ribulose - 1,5-bisP + CO₂ → 2. 3-phospho-erythrose

Nitrogen metabolism (ch. 18)
Nitrogen usually not stored (like carbohydrates, lipids)
Many microorganisms can synthesize all necessary amino acids.
Mammals can synthesize some, but not all, amino acids.
Some must be obtained from food = essential amino acids (see Table 18.1)
Shortage of some amino acids for vegetarians, these are lysine, methionine, tryptophan.

Global nitrogen turn-over (see figure canvas: "key processes & microbes")
- N₂-fixation: only bacteria
- Nitrification: only bacteria, called chemolithotrophs; oxidation of NH₃ & NO₃⁻ for ATP-production
- Denitrification: only bacteria, anaerobic respiration with NO₃⁻, NO₂⁻ as alternative e⁻-acceptor, forming N₂O, NO, N₂:
  Important during waste-water treatment.
- Dissimilatory nitrate reduction: only bacteria, anaerobic respiration but with NH₃ as end product.
- Assimilatory nitrate reduction: most plants and microorganisms; reduction of nitrate to be incorporated as nitrogen in biomass
- NH₃ assimilation/excretion: most organisms; incorporation of nitrogen in biomass or excretion.

N₂ fixation
Converting N₂ into NH₃: N₂ → NH₃
Almost all nitrogen available are produced via this bacterial process
Only bacteria such as:
  - blue green bacteria
  - azotobacter
  - rhizobium: symbionts in root nodules of pea plants can be used as natural fertilization under "exchange farming"
  - klebsiella
Reaction catalyzed by enzyme nitrogenase, which is oxygen sensitive (irreversibly inhibited by O₂ exposure)
Protective mechanisms to avoid O₂ exposure.
  - very fast respiration rate to keep O₂ low.
  - slime larger on cell surface to avoid O₂ diffusion.
  - nitrogenase localized to special cell types (heterocysts in blue-green bacteria) 10 where O₂ cannot reach

Incorporation of NH₃
Most common routes; see fig. 18.5
Most of assimilated NH₃ goes via glutamate/glutamine.
Transaminases (aminotransferases) transfers amino group, most commonly from glutamate to other carbon skeletons to produce other amino ac.
Glutamate + α-ketoacid ⇄ α-ketoglutarate + amino acid,
Important process both during biosynthesis and degradation of amino acids
Glutamate dehydrogenase (GDH)

Reductive amination of α-ketoglutarate.

\[ \alpha\text{-ketoglutarate} + \text{NH}_3 + \text{NAD}(PH) \rightarrow \text{glutamate} + \text{NAD}(P) \]

Relatively high \( k_m \) for \( \text{NH}_3 \sim 1 \text{mM} \)

Glutamate synthase (GOGAT)

exists in plants, but not other higher eukaryotes.

\[ \alpha\text{-ketoglutarate} + \text{glutamine} + \text{NAD}(PH) \rightarrow 2 \text{glutamate} + \text{NAD}(P) \]

Glutamate synthetase (GS)

\[ \text{Glutamate} + \text{NH}_3 + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + \text{Pi} \]

Regulation: 1) Autoregulatory feedback inhibition via cumulative feedback inhibition.

Partial inhibition by 8 metabolites (tryptophane, histidine, glucoseamine-6-P, carbamoyl phosphate, CTP, AMP, alanine, glycerine). If all 8 are present simultaneously at high concentration, the enzyme is almost completely inhibited.

2) Covalent modification via adenylation. 12 adenylation sites, partial inhibition of each one, almost completely inhibited if all 12 sites are adenylation.

Asparagine

\[ \text{aspartate} + \text{ATP} + \text{NH}_3 \rightarrow \text{asparagine} + \text{AMP} + \text{PP}_i \]

+ glutamine + glutamate

uses either NH\(_3\) or glutamate

Preference for glutamine

Carbamoyl phosphate synthetase

\[ \text{NH}_3 + \text{HCO}_3^- + 2\text{ATP} \rightarrow \text{carbamoyl phosphate} + 2\text{ADP} + 2\text{P}_i \]

\[ \text{glutamine} + \text{enzyme} \rightarrow \text{enzyme} + \text{glutamate} \]

enzyme for both these reactions
Protein turn-over
continuous biosynthesis and degradation of proteins "half time" for proteins.

Degradation of proteins amino acids via determination, excretion of amino groups and degradation of carbon skeleton via TCA-cycle

Amino acid + α-ketoglutarate → carbon skeleton - degraded via TCA-cycle (fig 18.12)

glutamate → α-ketoglutarate + NH

☆ NH₃ excreted as uric acids (bird, land living reptile, insects)
or as urea (mammals)

Skipping lipid Metabolism, not on the exam
The DNA molecule consists of two strands in opposite direction which are held together by hydrogen bonding between the nitrogen bases.

- A binds T with two bonds
- G binds C with three bonds

Sequence of DNA and RNA is ALWAYS read in the 5' to 3' direction. Ex: 5' GATCCAAAT 3'

Ch 22: How is DNA replicated
Ch 21: How is DNA arranged
Ch 23: How is DNA repaired and rearranged

DNA and RNA is elongated by attachment of an incoming nucleotide on the 3' end of an existing strand, using a complementary stand as template.

DNA - polymerase catalyzes the addition of dNTP to an existing 3' OH

Okazaki fragment: the part that fix the problem of lagging strand

Helicase: enzyme that "unwraps" DNA

Topoisomerases: relieves the tension of the DNA.

Single strand binding protein (SSB): keeps DNA in unwound form and bases in correct alignment.
Primase makes the RNA-primer used to initiate the DNA synthesis.

Remember that DNA-polymerase can only elongate an existing 3' end.

DNA-polymerases III (δ & ε in eukaryotes): elongates the RNA-primer if it is processed; synthesizes many bases before falling off. This is due to the presence of a "clamp" which attaches the enzyme to the DNA.

DNA polymerase I: removes RNA-primer and replaces with DNA. It also performs "nick translation".

RNA-primer can also be removed by RNase H.

DNApol I also has 5' exonuclease activity.

3' exonuclease activity of DNA-polymerases performs "proofreading".

Ligase links the Okazaki fragment.

*Check the slides on canvas "DNA lecture 1"*

In E. coli: DnaA-protein uses ATP-hydrolysis to "open" the double helix at the origin of replication.

Cell cycle: The process between when a cell has just divided until it's ready to divide again.
More about DNA

Haploid = one copy of DNA/cell (we have 3 billion base-pairs)
Diploid = two copies of DNA/cell (we have 2 x 3 billion base-pairs)

The number of genes differ with maybe a factor 10 between humans and the rest. It's just the amount of cells that differ. See fig in slides, fig 21.3

In fig 21.4 the x-axis is logged.

Exons = codes protein
Introns = no coding protein

Some of the rest DNA is actually been show that they help with translation of RNA, this is called the putative function.

The eukaryotic cell is much more complicated than a prokaryotic cell.

2 meters of DNA/cell is in nucleus (and also some in the mitochondria)
⇒ The average human body contains 16 to 32 billion km of DNA distributed among trillions of cells.

Eukaryotic DNA is wound around Histone Octamers, which are in turn organized via Histone H4 and non-histone protein.

During normal conditions, DNA is organized into chromatin fibers

The chromatin fibers can organize further into condensed chromosomes

DNA synthesis and partitioning occurs at specific times in the cell cycle.
Order of mitosis:
1) Interphase \( \{ G_1, S, G_2 \text{-phase} \} \) (these names isn't required to learn)
2) Prophase
3) Metaphase
4) Anaphase
5) Telephase and cytokinesis

Cell-cycle dependent kinase (CDK) in combination with cyclins regulate progression through cell cycle.
**Introduction, thermodynamics and Metabolism (Ch 3, 8-11)**

**Catabolism** (Degradation, formation of ATP)

**Metabolism** → **Anabolism** (Biosynthesis, requires ATP)

**ATP** short time storage of energy.

**Hydrolysis of ATP**: \[ \text{ATP} \rightarrow \text{ADP} + P_i \]

\[ \Delta G^{\circ} = -32.2 \text{ kJ/mole} \]

0 = standard condition = all concentrations = 1 mol

\[ \text{pH} = 7 \] or \[ [H^+] = 10^{-7} \text{ Mole} \]

\( \Delta G \) _{in vivo} at cellular concentrations ~ -50 kJ/mole

because \([\text{ATP}]\) usually higher than \([\text{ADP}]\)

\( \Delta G \) depends on:

a) type of reaction

b) Substrate - Product concentrations

**High energy compounds = Degradation of such compound release enough energy to produce 1 ATP from ADP + P_i** (See Tab 3.5)

**ATP hydrolysis** often "drives" other energy requiring reactions and the phosphate group is transferred to another molecule.

**Ex:** Glucose + ATP → Glucose - 6-phosphate - ADP

Glucose + P_i → Glucose - 6-P (Energetically impossible)

but

Glucose - 6-P → Glucose + P_i is possible and takes place during gluconeogenesis
Efficiency / Energy yield

Distribution between catabolism and anabolism differs depending on organism, type of metabolism, carbon and energy source. The more ATP produced in catabolism the larger the function of anabolism. Reflected in biomass yield (amount of biomass produced per amount of substrate consumed) g/g, g/mole

Ex: Baker’s yeast Saccharomyces cerevisiae can respire and ferment glucose

Fermentation: C₆H₁₂O₆ → 2C₂H₅OH + 2CO₂ 2ATP/glucose
Biomass yield ~ 0.1 g/g

Respiration: C₆H₁₂O₆ + 6O₂ → 6CO₂ + 6H₂O 28 ATP/glucose
Biomass yield ~ 0.5 g/g

\[ \Delta H = -100 \text{ kJ/mole} \]
\[ \Delta H = 1357 \text{ kJ/mole} \]
\[ 2 - 1357 + 100 = -2814 \text{ kJ/mole} \]
\[ \Delta H = -2814 \text{ kJ/mole} \]
ΔH state function = Path is not important only start and end production

Enthalpy change per ATP produced:
- Fermentation: -100 kJ/mole 2ATP → -50 kJ/mole ATP
- Respiration: -28141 kJ/mole 8ATP → -100 kJ/mole ATP
Metabolism and chemical redox reactions

Most organisms obtain energy by oxidising an organic substrate.

**Ex:** Oxidation of glucose

\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \]

Electrons are removed from glucose to \( O_2 \) and \( H_2O \) is formed.

Glucose: electron donor and oxidised

\( O_2 \): electron acceptor and reduced

But electrons are not transferred directly to \( O_2 \), instead co-enzyme are used.

**Ex:**

\[ \text{Co-enzymes} \]

**NAD/NADH:** \( NAD + 2e^- \rightarrow NADH \)

(*Always 2e^-, mainly in catabolism*).

**NADP/NADPH:** \( NADP + 2e^- \rightarrow NADPH \)

(*Always 2e^-, mainly in anabolism*).

**FAD/FADH:** \( FAD + 2e^- \rightarrow \text{semiquinone} + 2e^- \rightarrow FADH \)

(2e^- can be transferred directly to FAD without producing semiquinone).

Reduction potential is a measurement of a compound's affinity for electrons or tendency to become reduced or oxidised.

Low reduction potential \( \Rightarrow \) easily oxidised \( \text{ex NADH} \)

High reduction potential \( \Rightarrow \) easily reduced \( \text{ex O}_2 \) \( (O + 2e^- \rightarrow H_2O) \)

Energy released during oxidation depends on the difference in reduction potential between e^- donor and e^- acceptor.

\[ \Delta G^0 = -nF\Delta E^{0\circ} \]

\( n = \) number of electrons

\( F = \) Faraday's Constant

\( \Delta E^{0\circ} = \) Difference in reduction potential between e^- donor and e^- acceptor
NAD/NADH redox potential - 0.32 V  e^- acceptor
FAD/FADH redox potential - 0.22 V

NADH provides more energy than FADH

Respiration of glucose can be divided into two parts.
1) \( C_6H_{12}O_6 + 10 NAD + 2 FAD + 6 H_2O \rightarrow 6 CO_2 + 10 NADH + 10 H^+ + 2 FADH_2 \)
   Glycolysis, PDH, TCA cycle release 24 e^-

2) 10 NADH + 10H^+ + 2 FADH_2 + 6O_2 \rightarrow 10 NAD + 2FAD + 12H_2O
   electron transport chain 24 e^- released from NADH, FADH
   and O_2 reduced to H_2O

Fermentation process are redox neutral no net oxidation or reduction yet ATP production is possible.

Classification of organisms

Organisms can be classified according to type of
A) Energy metabolism
   A1) Phototrophs (Light as energy source, green plants, cyanobacteria and some other bacteria)
      A1.1) Photolithotroph (inorganic e^- donor)
      A1.2) Photoorganotroph (organic e^- donor) (only some bacteria)
   A2) Chemotrophs (chemical redox reactions as energy source)
      (animals, most bacteria)
      A2.1) Chemolithotrophs (inorganic e^- donor) (some bacteria)
      A2.2) Chemoorganotroph (organic e^- donor)
Also e⁻-acceptor can differ:
A2.a) External
   A2.a.1) Oxygen (aerobic respiration) (animals, most bacteria)
   A2.a.2) Not oxygen. NO₃⁻, SO₄²⁻ etc. (anaerobic respiration (some bacteria)

A2.b) No external e⁻-acceptor
   Fermentation

B) Depending on carbon source
   B1) Heterotrophs (organic compounds) (animals, most bacteria)
   B2) Autotrophs (CO₂) (Green plants, cyanobacteria)

Classification of enzymes
Standardization by an international agreement (IUBMB), 6 main classes
1) Oxidoreductases, oxidation-reduction reactions
2) Transferases, transfer a group from one molecule to another
3) Hydrolases, hydrolytic cleavage
4) Lyases, removes or adds a group to a double bond
5) Isomerases, intramolecular changes
6) Ligases, joins two molecules

Each enzyme is given a unique number by Enzyme Commission within IUBMB. EC = Enzyme commission

EC 1.1.3.5

Traditional names
Dehydrogenases, oxidation-reduction process often with NAD(P)H or FADH as co-enzymes
Oxidases, O₂ e⁻-acceptor
Oxygenses, O₂ is incorporated to a substrate that becomes oxidised
Carboxylases - Decarboxylases, CO₂ is added or removed from a molecule
Isomerases - Mutases, intramolecular changes
Kinases and phosphatases, add or remove a phosphate group from a molecule or protein

Regulation of rate or flux through biological pathways

DNA → mRNA → Proteins → Pathways
A) Regulation via amount of protein
   Induction - Repression, see Lac operon and Trp operon
B) Regulation via changes in activity of proteins
   - Substrate and Product concentrations see fig 8.21

- Allosteric regulation
  a) Homoolosteric regulation, (Cooperative substrate binding)
     Enzymes with several binding sites for substrate and when one substrate molecule binds the affinity in the remaining sites increases dramatically. Ex: Hemoglobin
  b) Heteroolosteric regulation
     Enzyme with binding sites (not substrate binding site) for effector molecules that either activates or inhibit activity of the enzyme. See fig 8.38
     Molecules are bound in "pockets" within the enzyme via hydrogen bonds, van der Waals forces not covalently

\[
\begin{array}{c}
\text{V} \\
\text{V}_{\text{max}} \\
\frac{1}{2} \text{V}_{\text{max}}
\end{array}
\]

\[
\begin{array}{c}
k_m \\
[k]
\end{array}
\]

Presence of activator and inhibitor affects km but not vmax

\[
\begin{array}{c}
\text{V} \\
\text{V}_{\text{max}} \\
\text{V}_{\text{max}}
\end{array}
\]

\[
\begin{array}{c}
k_m \\
k_m \\
k_m
\end{array}
\]

+ activator
+ inhibitor
original
Feedback inhibition: \[ A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \]

End product in a pathway inhibits an enzyme in the beginning of the same pathway.

Feed-forward activation: \[ A \rightarrow B \rightarrow C \rightarrow D \]

An intermediate activates a protein further down in the same pathway.

Covalent modification

**Ex:** phosphate, adenylyl, acetyl group binds covalently to certain amino acids in the enzyme and affects its activity.

The most common modification is phosphorylation by protein kinases and dephosphorylation by protein phosphatases.

Phosphorylations are common during signal transduction.

i.e. When a signal from a hormone or environment should result in an intracellular response.

**Signal hormone:** First messenger (binds receptors on cell surface)

**C-AMP, Ca^{2+}** = Second messengers (initiates intracellular response)

Cascade of phosphorylations and dephosphorylations before activation of final target. See Glycogen mobilization fig 12.26

**Distributed control of metabolism (p.893)**

**Rate limiting enzyme:** One enzyme is rate limiting and determines the rate through the entire pathway.

**Ex:** Phosphofructokinase in glycolysis

**MCA = Metabolic Control Analysis**, All enzymes in a pathway are involved in rate regulation but to different extents.

Each enzyme is given a flux control coefficient, \( c^j \), between 0-1 that indicates its importance in flux regulation.

Sum of all \( c^j \) in a pathway = 1

If a rate limiting enzyme exists this will have \( c^j = 1 \), all other enzymes have \( c^j = 0 \).